Characterization of *Quercus* Species Distributed in Jordan Using Molecular Markers

Mohammad S. Jawarneh[1], Mohammad H. Brake[2], Riad Muhidat[1], Hussein M. Migdadi[3], Jamil N. Lahham[1], Ahmad Ali El-Oqlah[1]

1 Department of Biological Sciences, Faculty of Science, Yarmouk University

2 Science Department, Faculty of Science, Jerash University

3 Plant Production Department, Collage of Food and Agricultural Sciences, King Saud University, Saudi Arabia

Abstract

Genetic diversity among 25 natural populations of three different species of *Quercus* in Jordan at molecular levels using Random Amplified Polymorphic DNA (RAPD) primers was assessed. Significant molecular variations among and within 25 *Quercus* populations were estimated. Twenty-seven polymorphic markers and 5917 scored bands were generated using six RAPD primers. Based on RAPD data, the populations were grouped together in the same cluster according to species regardless to local of collections. This study has emphasized the ability of the molecular markers in the determining the genetic diversity among and within the populations of *Quercus* and the resulted high genetic variability could be utilized in implications of improving conservation, restoration, and reforestation strategies of *Quercus* in Jordan.

Keywords: Quercus spp, genetic diversity, RAPD markers, conservation, restoration

1. Introduction

Quercus L. (Oak) is one of the exceptionally important woody genera worldwide. It is a large genus in the family Fagaceae with about 600 species growing in a wide range of habitats and distributed in temperate and subtropical regions of the northern hemisphere (Yilmaz et al. 2011). Members of the genus grow as shrubs and trees and form prominent deciduous forests or evergreen woodlands with a range of distribution extending from cold latitudes to tropical Asia and the Americas (Manos et al. 1999).

In Jordan, the genus constitutes an important component of the forest ecosystems in the Mediterranean topographic zone. Three species of the genus are known to occur naturally in this region with a general range of distribution extending from Aum Qais in the North to Tafilah in the South. These species are *Quercus calliprinos L., Quercus ithaburensis, and Quercus infectoria* (Kasapligil 1956, Long 1957, Zohary 1961; 1962; 1973). *Q. calliprinos* is the most widespread and evergreen species; distributed throughout the Mediterranean region from Ajloun in the North through Salt and Fuhais to Tafilah and Shobak in the South. *Q. infectoria* is the least abundant and deciduous species; restricted largely to the Northern parts of the country. *Q. ithaburensis* is intermediate in terms of distribution and abundance; it forms deciduous forests in the Northern

and middle parts of the country, mainly around cities of Irbid, Jarash, Salt, and Fuheis. Information on levels of genetic diversity within and among populations of Quercus species in Jordan per se is lacking. Knowledge of the genetic variation of this important genus provides a robust framework for follow-up systematic studies and facilitates its use in genetic conservation and rehabilitation. In addition, this information will help understand the dynamics of the population genetics of *Quercus*, its evolutionary trends, and its responses to changes in the environment.

In this study, the classical technique of random amplified polymorphic DNA (RAPD) is employed to investigate for the first time levels of genetic diversity of natural populations of the genus *Quercus* in Jordan. In Palestine the *Quercus boissieri* Reut. an associated species within the *Quercus calliprinos*–Pistacia palestine association of the Mediterranean sclerophyllous broad-leaf forests. The use of random amplified polymorphic DNA (RAPD) markers,within- and among-populations genetic diversity of *Quercus boissieri* in Palestine , as influenced by geo-climatic parameters (Schiller, et al., 2006).

2. Materials and methods

Samples of the three different Jordanian *Quercus* species were collected from the field during October, November and December of 2008. Samples were randomly selected from different populations distributed over various geographical regions in Jordan. Samples were identified cautiously and taxa names were confirmed following Zohary, (1962). Fresh leaf samples were stored at -20 for DNA extraction.

Leaves collected from each individual tree were manually ground in liquid nitrogen with a mortar and pestle, to a fine powder and DNA was extracted according to the protocol of Genomic DNA Purification Kit form Fermentas and then the DNA samples were stored at -20ºC until use.

Six DNA samples were tested using 60 primers from Operon kits A,B and D. Primers that amplified consistently reproducible polymorphisms were selected and used to analyze all of the 25 *Quercus* populations. Only six RAPD primers were used to amplify the 25 populations.

RAPD reactions were performed in total volume of 15 μl according to standared protocol (Sambrook *et al.*,1989). The amplification products were loaded using 1.8% agarose gel electrophoresis at 100 volts for 2 hrs using horizontal gel electrophoresis apparatus. The amplified products were visualized and documented by gel documentation system. 100 bp ladders were used as a DNA marker to estimate the molecular weights of the amplified products.

Data generated from RAPD analysis were analyzed using Jaccard similarity coefficients (Jaccard, 1908). These similarity coefficients were used to construct dendrograms using the unweighted pair group method with arithmetic average (UPGMA) employing SAHN (sequential, agglomerative, hierarchical, and nested clustering) using the NTSYSpc (ver.2.10) program, (Rohlf, 2005).

3. Results

A total of 60 RAPD primers were evaluated for their ability to amplify polymorphic regions from six randomly selected populations. Of the 60 primers, 6 amplified consistently reproducible polymorphisms, and so these were used to analyze all of the 25 *Quercus* populations. The features of the primers across the tested populations are summarized in Table 1. The 6 primer

generated a total of 27 polymorphic markers (alleles). In total, 5917 data points (bands) could be scored with an average of 986.2 bands per primer pair across the genotypes, thereby confirming the high multiplex ratio expected for the RAPDs. The ability of different primer to generate RAPD markers varied from 4 to 5 markers with an average of 4.5 markers per primer pair across all genotypes. On a per-population basis, the number of markers generated by the primer pairs ranged from 32.4 for OPD 17 to 47.9 for OPA 20 with an average of 39.4 markers per primer. The percentage of polymorphic among the primers generated was100% polymorphic markers.

Polymorphic bands ranged in size from 250 to 790 bp. The size out of this range was not considered in the analysis). The densely stained markers were considered in scoring. The total bands for each primer ranged from 810 for primer OPD-17 to 1197 bands for OPA-20 using 389 plants representing 25 *Quercus* populations.

Primer	Total markers[a]	Average bands[b]	Polymorphic markers[c]	Polymorphic markers %	Size range (Bp)	Total no. of bands[d]
OPA 12	4	43.4	4	100	280-700	1086
OPA 17	5	42.0	5	100	250-750	1050
OPA 19	4	35.5	4	100	300-790	887
OPA 20	5	47.9	5	100	250-680	1197
OPB 5	4	35.5	4	100	400-780	887
OPD 17	5	32.4	5	100	380-750	810
Total	27		27			5917
Average	4.5	39.4	4.5	100		986.2

Table 1. The features of RAPD primers selected in *Quercus* genetic diversity

a Total number of differently sized RAPD markers amplified across all 25 populations, b Average number of RAPD bands scored per population, c Total number of RAPD markers found to be polymorphic across the 25populations, d Total number of RAPD bands (data points) scored for all populations

Based on the Jaccard coefficients index (Jaccard, 1974), a genetic similarity matrix was constructed using the RAPD data to assess the genetic relatedness among the 25 *Quercus* populations. The within population means were used to construct the similarity matrix. The mean similarity indices ranged from 0.24 between population 4 and population 11 to 0.84 within the population number 8 and 0.48 for over all populations.

The results showed based on RAPD product data that populations from the different locality represent species tend to grouped together in the same cluster figure (1). The species of the 25 *Quercus* populations clustered into two main clusters; the first cluster consists of the populations belong to *Quercus ithaburensis* and the second cluster consists of the populations belong to the *Quercus infectoria* and *Quescus Calliprinos* species.

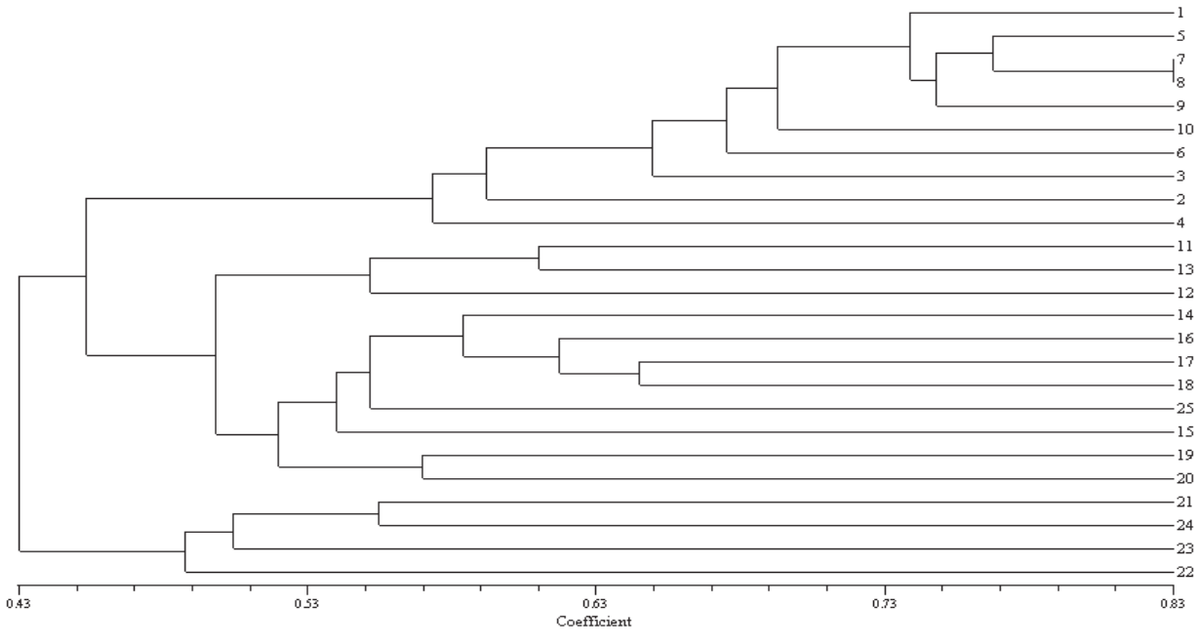

Figure 1. Dendrogram of 25 *Quercus* L. (1=Ber Adbagat, 2= Al-Rashadia, 3= Heisha, 4= Dana,5= Achtifina, 6=Ebein, 7= Enbeh,8= Anjara, 9= Rahaba,10= Fuheis,11= Gelad, 12= Kufour Houda, 13= Zobia, 14= Bargesh 15= Ebein, 16= Ashah, 17= Umm Qaiss, 18= Aosra, 19= Jeneen Safa, 20= Aqraba, 21= Kufour Kifya, 22= Makhraba, 23= Alouk, 24= Jobbah, 25= Gelad) generated by UPGMA cluster analysis of the genetic similarity values.

4. Discussion

The 25 populations analyzed in this study represented *Quercus* species from a wide range of geographical areas in Jordan**.** In this work we followed the nomenclature used in the previous workers (Kasapligil 1956, Long 1957; Zohary 1962, 1973). Our results showed that Jordan has at least three *Quercus* species and each has its morphological characters.

The current study uses the RAPD-PCR based protocol to assess genetic variability of the *Quercus* species in Jordan. Genetic diversity determines the adaptive potential of a species and is an essential component of the stability of ecosystems. Analysis of within- and among-population genetic diversity is a fundamental step in the development of strategies for conservation of genetic resources and, consequently, of their adaptability. With its oak forests that comprise *Quercus ithaburensis*, *Quercus boissieri*, and *Quercus calliprinos*, is in a geographically peripheral position to the main area of distribution of these species in the Mediterranean basin (Awishi, 1967). According to (Safriel et al. 1994), unlike core populations, peripheral ones may be tolerant to environmental extremes and changes because of their higher genetic variability, which has resulted from fluctuating selection. It is also likely that peripheral populations evolve resistance to extreme conditions; therefore, they should be treated as a biogenetic resource, to be used for rehabilitation and restoration of damaged ecosystems. Owing to their long life cycle, forest trees are among the species that cannot migrate or adapt quickly enough to cope with the rapid changes imposed on the environment by human activity, and this could create ecological and forest management problems. Thus, attention should be given to in situ and ex situ conservation of the varieties of

Quercus ithaburensis genetic material represented by the three main assemblages of its distribution in this region.

In this study it was possible to show that the amplification products from six random primer RAPD assay were sufficient to discriminate among and within population of *Quercus* species for each location. Also, the assay was useful in discriminating among plants of the same location. The ability to distinguish between closely related individuals was simply a function of the observed number of RAPD bands.The results of RAPD markers were compared in a genetic diversity of *Quercus* species the differences in the level of polymorphism detected by the markers and evaluating the potential of these markers in assessing the genetic variation in 25 population of *Quercus* to three species *Quercus ithaburensis*, *Quercus infectora*, and *Quercus calliprinos*. that matching result come into view the morphological result. The classes of molecular markers adopted in this study deserve additional discussion. The key of the success of multilocus PCR-based markers has to found in their high multiplex ratio. In fact, owing to their own genetic nature, RAPD assays detect simultaneously many loci randomly distributed in the genome. Moreover, compared to SSRs, these marker systems allow a more precise estimate of marker allele frequencies at single loci and faster estimate of population polymorphisms over several loci.

From the similarity matrix the highest values of similarity between populations was found between the populations Aqraba, Makhraba, Ashah and Umm Qiass these population are cluster together in the hierarchical cluster constructed on the base of the genetic similarity values. These population belong to the same species *Quercus ithaburensis* and the population are found in the same region and located at the same elevation. The results obtain confirm once again the great versatility, reliability and precision of the techniques based on molecular markers, which can be used to aid the classical evaluation of the differentiation between population based on the observation of morphological characteristics. Our molecular results also in agreement of those Cottrell et al. (2003) who used six microsatellite markers found high expected heterozygosity values in Quercus robur and Quercus petraea populations. ranging from 0.87 to 0.92 and from 0.76 to 0.82 in Q. crispula populations (Ohsawa et al., 2007d). And with those of SCHILLER et al 2003 who found that Quercus aegilops L. ssp. ithaburensis populations were aggregated according to main geographic regions.

In conclusion, the variations among *Quercus* species studied at molecular levels indicated that there is a high variation among these populations and the RAPD technique was useful for studying genetic variability of *Quercus*. The wide geographical distribution of *Quercus* populations across different environments means that this species has good genetic resources to fill the gap between northern natural distribution sites with the southern natural distribution site. *In-situ* as well as *ex-situe* conservation, restoration, and reforestation should be done in the nearest populations within the same geographic region.

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